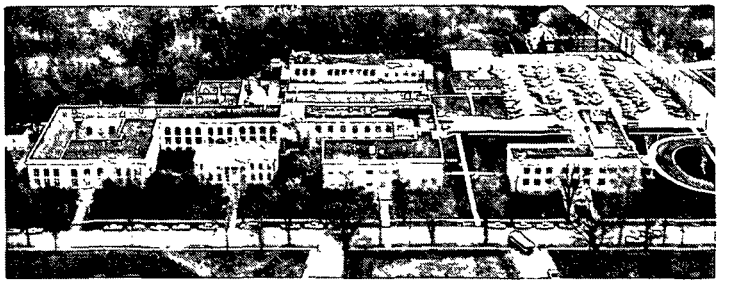

THE INSTITUTE OF PAPER CHEMISTRY, APPLETON, WISCONSIN

IPC TECHNICAL PAPER SERIES
NUMBER 53

MOLECULAR ORIENTATION IN PLANT CELL WALLS: DETECTION USING RAMAN SPECTRA OF INDIVIDUAL CELLS

RAJAI H. ATALLA AND REBECCA E. WHITMORE

MARCH, 1978

Rajai H. Atalla

MOLECULAR ORIENTATION IN PLANT CELL WALLS: DETECTION USING RAMAN SPECTRA OF INDIVIDUAL CELLS

INTRODUCTION

In the course of our programs directed at modification of fiber properties we have developed a number of new techniques for characterizing fiber structure. The method described in the present note, wherever we describe studies of the Raman spectra of single fibers, has been a key factor in our ability to interpret some of the changes which occur upon conversion from the cellulose I to the cellulose II form. The note outlines the new class of information that is accessible through this technique.

This paper has been submitted for publication in Science.

ABSTRACT

Techniques for recording Raman spectra of individual plant cells have been developed. Control of polarization of the incident laser beam and analysis of polarization in the scattered beam, relative to cell axes, have made possible determination of molecular orientation in the cell walls. The techniques promise to provide new information on cell wall architecture.

We report evidence for molecular orientation in cell walls derived from Raman spectra of individual cells. Evidence of molecular orientation in the cell walls of higher plants has, in the past, been confined to average measurements on groups of fibers; in x-ray diffractometry, axially aligned bundles of fibers (1,2), and for infrared dichroic measurements, arrays of fibers aligned parallel to one another in a plane perpendicular to the infrared beam (3). We report here spectra recorded for individual fibers and comment on the manifestations of molecular order in the spectra.

Although we have also recorded spectra on fibers from cotton and loblolly pine [Pinus taeda L.], we will highlight those recorded for ramie [Boehmeria nivea] fibers; ramie fibers are known to have the vast majority of the cellulose chains aligned parallel to the fiber axis. The samples of ramie were obtained from the collection kept at The Institute of Paper Chemistry by Dr. Irving Isenberg. They were subjected to mild bleaching, to reduce fluorescence in the laser beam, and were then mounted across small washers and placed in the 180° back-scattering sample accessory of a Spex 1401 Raman spectrometer. They were aligned in the laser beam such that their axes were in the same plane as the entrance slit of the spectrometer, and the image of the illuminated portion just filled the slit. Spectra were recorded with the polarization of the incident beam parallel and perpendicular to the axis of the fiber, and in each of these modes Raman scattered radiation was detected through an analyzer positioned to transmit light polarized parallel or perpendicular to the plane of polarization of the incident beam. Thus, both polarized and depolarized spectra were recorded with the electric vector of the exciting radiation parallel and perpendicular to the axis of the fiber.

Though in the spectrum of a polymeric system of C_1 symmetry all bands are expected to be polarized to some degree, it is anticipated that highly polarized bands reflect transition moments where the terms arising from the symmetric components of the polarizability tensor are dominant. If such bands are associated with motions in a particular system of bonds, and if they are found sensitive to the relative orientation of the electric vector of the exciting radiation, they can provide an index of the degree of orientation of the particular system of bonds.

Two key regions of spectra are shown in Fig. 1. These are the 800-1500 cm^{-1} region associated primarily with skeletal stretching motions and with methyl and methine deformations, and the 2800-3000 cm^{-1} region associated with the CH-stretching vibrations. The three spectra shown are: (a) the polarized spectrum with the incident electric vector parallel to the axis of the fiber, (b) the polarized spectrum with the incident vector perpendicular to the axis of the fiber, and (c) a depolarized spectrum. Only one depolarized spectrum is included because the two were identical.

The bands of primary interest are the one at 1095 cm^{-1} and the broader one centered at approximately 2920 cm^{-1}; these are the bands most sensitive to polarization of the incident beam. The first, the most intense of the skeletal bands, has been associated with the symmetric stretching of an essentially coplanar system of bonds including the glycosidic linkage as well as components of neighboring anhydroglucose units; a vector through the centers of these bonds is essentially parallel to the axis of the cellulose chains. The 2920 cm^{-1} bands have been associated with methine CH stretches. Since in cellulose all of the heavy atoms attached to the pyranose rings are equatorial, all of the methine CH bonds are axial and, hence, perpendicular to the axis of the chain.

The high degree of polarization and the sensitivity to the orientation of the incident electric vector suggest that the primary contributors to intensity are the symmetric components of the polarizability tensors. The response to rotation of the plane of the incident vector can thus be taken as a measure of the inclination of the transition moment with respect to the incident vector.

The interpretation of the spectra shown for ramie is consistent with the spectra recorded for other fibers wherein the average orientation of the molecules relative to the cell axes are known. For cotton, where the molecular orientation is generally at approximately 45° to the fiber axis, the spectra for the two orientations of the incident polarization are almost identical. The results for loblolly pine fibers, where molecular alignment in the direction of the axis is somewhat greater than in cotton, the sensitivity of the polarized bands to the orientation of the incident electric vector is intermediate between that observed for ramie and that observed for cotton.

While the spectra in Fig. 1 were recorded with the fibers mounted parallel to the entrance slit, we have recently obtained similar spectra with a Ramanor double monochromator and with the cells mounted such that their axes were perpendicular to the plane of the entrance slit; translation of the fibers parallel to their axes permitted exploration of axial variations in molecular order and, in consequence, closer examination of cell wall architecture.

Although the spectra recorded so far do not indicate significant departures from the previously reported average measurements, they do suggest a degree of intrafiber and intraspecies variability. For example, the polarized bands at approximately 1460 cm^{-1}, which appear of equal intensity in both polarized spectra of the particular fiber, occur with different relative intensities

or with much reduced intensities in the spectra of other fibers. A number of other variations in the spectra have also been noted, but they remain to be systematized.

The major point of the present report is that Raman spectra of individual cells can be recorded in a manner which reveals details of wall architecture previously beyond detection with nonintrusive methods.

Rajai H. Atalla
Rebecca E. Whitmore
The Institute of Paper Chemistry
Appleton, Wisconsin 54911

REFERENCES

(1) R. D. Preston, The Physical Biology of Plant Cell Walls (Chapman and Hall, London, 1974), and references cited therein.

(2) A. Frey-Wyssling, The Plant Cell Wall (Gebruder Borntraeger, Berlin, 1976), 3rd edition, and references cited therein.

(3) J. Blackwell and R. H. Marchessault, in Cellulose and Cellulose Derivatives, Part IV, N. M. Bikales and L. Segal, Eds. (Wiley-Interscience, New York, 1971), and references cited therein.

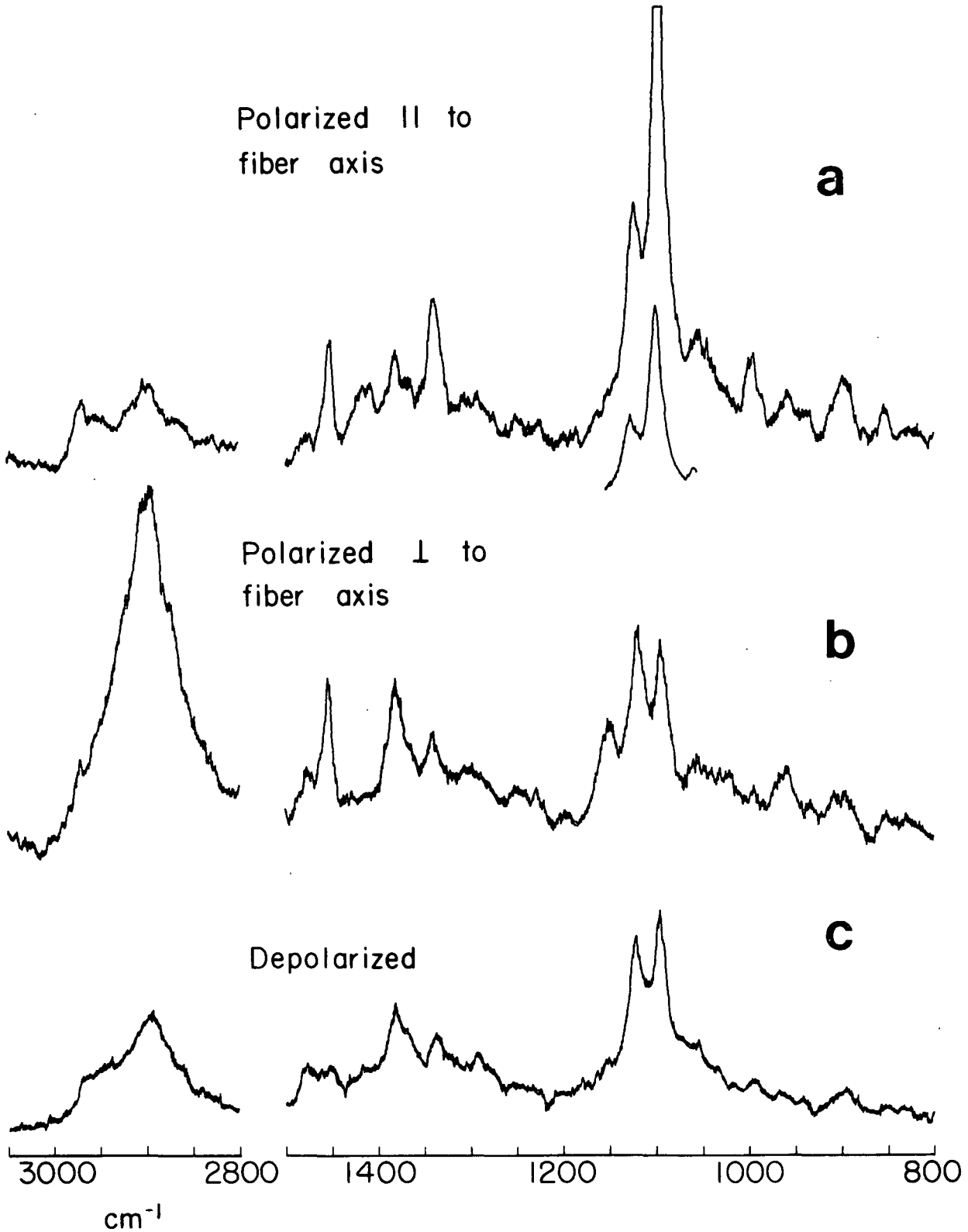

Fig. 1. Raman spectra of part of a ramie fiber approximately 250 μ long.